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Formulation and Antibacterial Activity of *Plectranthus amboinicus* (Lour.) Spreng Leaves Ethanolic Extract as Herbal Mouthwash Against Halitosis Caused Bacteria

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Keywords: Antibacterial activity; Ethanolic extract; Halitosis; Mouthwash; Plectranthus amboinicus (Lour.) Spreng

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Abstract

BACKGROUND: Halitosis is one of the unpleasant conditions which could alter the self-confidence and cause serious illness. One of the treatments to overcome halitosis is by using mouthwash. *Plectranthus amboinicus* (Lour.) Spreng is one of the plants that is known to have antibacterial activity which can be used to overcome halitosis caused bacteria.

AIM: To formulate the *Plectranthus amboinicus* (Lour.) Spreng leaves ethanolic extract as herbal mouthwash and to evaluate the antibacterial activity against *Streptococcus mutans* and *Staphylococcus aureus* bacteria.

METHODS: The methods of the study included the characterization and screening of *Plectranthus amboinicus* (Lour.) Spreng leaves dried powder; the extraction process by maceration using 96% ethanol; the antibacterial activity test of *Plectranthus amboinicus* (Lour.) Spreng leaves ethanolic extract and the formulation and evaluation of mouthwash dosage form with various concentrations of *Plectranthus amboinicus* (Lour.) Spreng leaves ethanolic extract.

RESULTS: The characterization results showed that the *Plectranthus amboinicus* (Lour.) Spreng leaves powder contained 7.92% of water, 29.26% of water-soluble substance, 13.32% of ethanol soluble substance, 0.82% of total ash and 0.66% of acid insoluble ash. The screening examination gave glycoside, saponin, flavonoid, tannin and steroid/triterpenoid positive results. The antibacterial activity test of the *Plectranthus amboinicus* (Lour.) Spreng mouthwash showed that the dosage form with 2% of *Plectranthus amboinicus* (Lour.) Spreng extract successfully inhibited the growth of bacteria with 12.00 and 11.25 mm of inhibition diameter for both *Staphylococcus aureus* and *Streptococcus mutans*, respectively.

CONCLUSION: The *Plectranthus amboinicus* (Lour.) Spreng leaves ethanolic extract can be formulated in mouthwash dosage form and has antibacterial activity against *Staphylococcus aureus* and *Streptococcus mutans* bacteria.

Introduction

Halitosis is an unpleasant oral odour, emerging from the oral cavity whereby if it is prolonged untreated can decrease the self-confidence and cause serious illness [1], [2]. Approximately 80% of all halitosis cases are caused by the bacterial degradation of organic substrates left in mouth, which mostly resulting volatile sulphuric compound [2], [3]. The microorganisms that usually found as the cause of halitosis related diseases such as dental caries were *Streptococcus mutans* and *Staphylococcus aureus* [4], [5]. One of the treatments of halitosis is oral hygiene by using mouthwash. The utility of mouthwash containing antibacterial to treat bad breath has been suggested to kill the bacteria, to rinse the debris of food and to lift the plaque in the oral cavity [6].

Mouthwash is a solution that containing breath refreshment substance, adstringent, demulcent, surfactant or antibacteria to refresh and clean the airways whereby the usage is by rinsing [7]. The active substance as antibacterial can be obtained from the nature, hence it is called herbal mouthwash [4]. In this research, *Plectranthus amboinicus* (Lour.) Spreng leaves ethanolic extract was used as the active agent. *Plectranthus amboinicus* (Lour.) Spreng

leaves was reported to have volatile compounds such as carvacrol, linanol, geranyl acetate, γ-terpinene, *p*-cymene, nerol, α-4-carene, caryophyllene and β-myrcene [8]. The powder leaf of *Plectranthus amboinicus* (Lour.) Spreng also contained non-volatile compounds which had been reported by previous studies such as flavonoids, terpenoids, saponins, steroids, tannins, proteins, and carbohydrates [9], [10], [11]. The compounds in *Plectranthus amboinicus* (Lour.) Spreng leaf showed antimicrobial activity against several microorganisms as highlighted in the previous reported studies [12], [13].

Therefore, this study was performed to formulate a mouthwash dosage form containing *Plectranthus amboinicus* (Lour.) Spreng extract as active ingredient to treat halitosis caused bacteria.

Material and Methods

Materials

The materials used in this study were *Plectranthus amboinicus (*Lour.) Spreng leaves, nutrient agar, nutrient broth, 96% ethanol, *Staphylococcus aureus* bacteria*, Streptococcus mutan* bacteria, saccharin*,* Tween 80, citrus oil, distilled water, commercial mouthwash, alpha naphtol, hidrochloric acid, acetic acid anhidride, glacial acetic acid, nitric acid, sulphuric acid, benzene, iron (III) chloride, bisthmuth (III) nitrate, ethanol, ethyl acetate, n-hexane, iodine, isopropanol, potasium iodide, chloroform, methanol, sodium hidroxide, sodium chloride, sodium sulphate anhydrate, mercury (II) chloride, magnesium powder, lead (II) acetate and toluene. All of the chemical reagents used were analytical grade.

Methods

The *Plectranthus amboinicus (*Lour.) Spreng leaves were washed and dried at temperature of 40°C for 3 days. The dried leaves were powdered, then the powder was characterized and screened for the the phytochemical compound content. The extract was prepared by maceration method using 96% ethanol [14]. The extract was concentrated using rotary evaporator until a viscous extract was obtained. The obtained extract was called as ethanolic extract of *Plectranthus amboinicus (*Lour.) Spreng leaves (EEPL).

Formulation of Mouthwash

The formula of mouthwash was varied according to the concentration of EEPL as shown in Table 1. The basic formula of mouthwash was adopted from Mitsui [15].

Table 1: The composition of mouthwash formula

Ingredients	F0	F1	F2	F3	F4
EEPL	-	0,75%	1%	2%	3%
Tween 80 pH 6-8 5%	12%	12%	12%	12%	12%
Saccharin	0,1%	0,1%	0,1%	0,1%	0,1%
Citrus oil	0,2%	0,2%	0,2%	0,2%	0,2%
Distilled water to	50 ml	50 ml	50 ml	50 ml	50 ml

F0: Blank (without extract); F1: Mouthwash with 0.75% EEPL; F2: Mouthwash with 1% EEPL; F3: Mouthwash with 2% EEPL; F4: Mouthwash with 3% EEPL.

Evaluation of Mouthwash

The evaluation of mouthwash stability included form, colour, odour and pH [16]. Mouthwash was stable if there were no changes in colour, odour, pH and appearance during the storage at room temperature. The observation was conducted at the 0, 1, 2, 3 and 4th week of storage.

Antibacterial activity test

The antibacterial activity test was performed for both EEPL and mouthwash dosage form. The antibacterial activity test was conducted using diffusion agar method as described in Valgas et al., [17]. The antibacterial activity was judged by measuring the inhibition zone diameter against *Staphylococcus aureus* and *Streptococcus mutans* bacteria after 24 hours incubation.

Results

Phytochemical Screening and Characterization of Plectranthus amboinicus (Lour.) Spreng leaves dried powder

The phytochemical screening evaluation of *Plectranthus amboinicus (*Lour.) Spreng leaves dried powder showed the positive result of chemical compounds including flavonoid, glycoside, saponin, tannin, and steroid / triterpene. This result was accordance with the result of the other previous study reported by Kaliappan and Viswanathan [9].

The characterization of the *Plectranthus amboinicus (*Lour.) Spreng leaves dried powder is shown in Table 2.

Table 2: The characterization results of *Plectranthus amboinicus (*Lour.) Spreng leaves dried powder

Parameter	Result (%)
Water content	7.92
Water soluble substance	29.26
Ethanol soluble substance	13.32
Total ash	0.82
Acid insoluble ash content	0.66

Antibacterial activity of EEPL

The antibacterial activity of EEPL was done to

find the optimum dose for mouthwash formulation. The test demonstrated that EEPL inhibited the growth of *Staphylococcus aureus* and *Streptococcus mutans* bacteria, indicated by the clear zone around the disk. EEPL had been succesfully inhibited the growth of *Staphylococcus aureus* and *Streptococcus mutans* bacteria started at concentration of 0.3 and 0.4%, respectively. At this concentration, EEPL gave inhibition zone diameter of 6.03 and 7.10 mm for *Staphylococcus aureus* and *Streptococcus mutans,* respectively. The effective inhibition was highlighted at concentration of 1.5% with inhibition zone diameter of 14.12 and 14.87 mm for *Staphylococcus aureus* and *Streptococcus mutans,* respectively. The diameter of the clear zone was elevated with EEPL concentration. The antibacterial activity of EEPL can be seen in Table 3.

Table 3: The antibacterial activity of EEPL against *Staphylococcus aureus* and *Streptococcus mutans* bacteria

Concentration	Inhibition zone diameter (mm)	
	Staphylococcus aureus	*Streptococcus mutans*
3.5%	22.75	17.22
3%	21.52	16.42
2.5%	19.43	15.54
2%	18.48	15.17
1.5%	14.12**	14.87**
1%	12.60	12.73
0.75%	10.30	10.77
0.5%	7.12	8.15
0.4%	6.17	7.10
0.3%	6.03	-
0.2%	-	-
Blank	-	-

- = no inhibition; ** = effective as antibacterial.

Evaluation of mouthwash dosage form

The mouthwash formula resulted a clear solution for the F0 as blank without EEPL. In the other hand, the formula containing EEPL showed a brownish green colour solution with specific odour as shown in Figure 1.

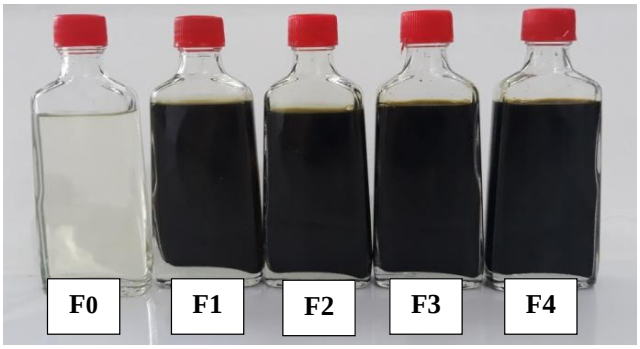

Figure 1. Mouthwash dosage form for each formula. F0: Blank (without extract); F1: Mouthwash with EEPL 0.75%; F2: Mouthwash with EEPL 1%; F3: Mouthwash with EEPL 2%; F4: Mouthwash with EEPL 3%

Stability of mouthwash dosage form

Based on the stability study that conducted for 28 days, it was demonstrated that all of the formula showed no changes in colour, odour and appearance. It indicated that the prepared dosage forms were physically stable.

The evaluation of pH was performed to determine the safety of mouthwash usage. Most of bacteria has optimum growth pH in the range of 6.5 – 7.5 [18]. Therefore, the pH of formulation should be out of the range of optimum pH of bacteria growth. The result of pH evaluation showed that either the blank and the herbal mouthwash had pH 5.87 – 6.00. The excipients added in the formula slightly decreased the pH during the storage of 28 days, since the excipients posessed acid properties. However, the decrease of pH was still in the range of acceptable criteria for mouthwash which is 4.0 – 6.5.

Table 5: pH evaluation result during storage

Day	pH					
	F0	F1	F2	F3	F4	Commercial mouthwash
0	6.03	6.00	6.00	5.90	5.87	5.8
7	5.97	5.87	5.83	5.73	5.70	5.8
14	5.90	5.77	5.73	5.67	5.63	5.8
21	5.77	5.67	5.57	5.53	5.50	5.8
28	5.57	5.47	5.37	5.33	5.30	5.8

Antibacterial activity of EEPL Mouthwash Dosage Form

Based on the antibacterial activity test of EEPL, the mouthwash dosage form was formulated at concentration of 0.75, 1, 2 and 3%. The antibacterial activity result of EEPL mouthwash dosage form formula against *Staphylococcus aureus* and *Streptococcus mutans* is presented in Table 4.

Table 4: The inhibition zone diameter of EEPL mouthwash dosage form

Formula	Inhibition zone diameter (mm)	
	Staphylococcus aureus	*Streptococcus mutans*
F0 (Blank)	-	-
F1	-	-
F2	8.13	-
F3	12.00	11.25
F4	13.42	13.22
Commercial mouthwash	8.35	9.5

F0: Blank (without extract); F1: Mouthwash with 0.75% EEPL; F2: Mouthwash with 1% EEPL; F3: Mouthwash with 2% EEPL; F4: Mouthwash with 3% EEPL.

The blank formula showed no inhibition activity for both bacteria. Surprisingly at concentration 0.75% in the mouthwash dosage form, there was no inhibition of bacterial growth for both *Staphylococcus aureus* and *Streptococcus mutans.* This result was different with the activity shown by EEPL alone. The formulation of EEPL in mouthwash dosage form had declined the antibacterial activity.

The inhibition zone was started to occur for F2 with 1% concentration of EEPL against *Staphylococcus aureus* only, otherwise the growth of *Streptococcus mutans* could be altered with higher concentration. Table 5 shows that F3 possesed inhibition zone with inhibition diameter 12.00 and 11.25 mm for *Staphylococcus aureus* and *Streptococcus mutans*, respectively. It also had higher inhibition zone diameter compared to the commercial mouthwash.

Discussion

The ability of EEPL in the mouthwash formula to decline the growth of tested bacteria demonstrated the effectivity of the herbal mouthwash to overcome halitosis. Some studies had identified the chemical compounds contained in *Plectranthus amboinicus* (Lour.) Spreng leaves which responsible for the antibacterial activity. Essential oil such as p-cymene, thymol, β-caryophyllene and γ-terpinene found in *Plectranthus amboinicus* (Lour.) Spreng leaves demostrated a positive result as antibacterial against *Staphyllococcus aureus* [19]. However, in this study the *Plectranthus amboinicus* (Lour.) Spreng leaves were extracted in ethanol solvent which produced most of the extracted coumpunds were non-volatile compounds. The non-volatile compounds of *Plectranthus amboinicus* (Lour.) Spreng leaves had been reported by Bhatt and Negi which mostly the phenolic compound to have antibacterial activity against the Gram-positive bacteria such as *Staphyllococcus aureus* [20]. In accordance with the result reported by Valgas, et al. and Gurgel, et al, *Plectranthus amboinicus* (Lour.) Spreng leaves extract showed a very potent result as antibacterial against *Staphyllococcus aureus,* due to the flavonoid compounds in the extract [17], [21].

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